Protein and sugar trends during the avocado season

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ABSTRACT

Ripening of avocado fruit is variable, being more rapid with increasing maturity, and within a consignment. Internal quality is often also variable. In order to understand the drivers behind the variability in fruit ripening and quality characteristics, the known ripening enzymes pectin methylesterase (PME), polygalacturonase (PG) and cellulase were studied during the ripening phase. Together with these enzymes, changes in ethylene and carbon dioxide evolution were monitored. Fruit was sourced from two climatic zones, Tzaneen and Howick, and across the season to evaluate the influence of maturity, which was estimated through measurement of moisture content. Ripening followed the ethylene climacteric, with more mature fruit ripening faster. Total proteins tended to follow the same pattern, but where initial concentration was high, a decline was not noted towards softening, while if low at harvest, proteins decreased towards softening. It is suggested that this is related to energy requirements for enzyme production, with the sugars mannoheptulose and perseitol showing sharp declines during the ripening. It is suggested that should these sugars not be sufficient to ensure enzyme production, then alternate energy sources, including proteins, will be utilised. Overall, total protein content at harvest as well as the C7 sugars in the fruit mesocarp could be an indicator of ripening and quality potential. Both can be measured by near infrared spectroscopy, allowing for prediction of ripening and quality. Further work will be needed to investigate the interaction with storage temperature.

INTRODUCTION

Avocados are climacteric fruit and substantial metabolic activity is required for ripening after harvest. The cell walls in the mesocarp need to be broken down to cause softening and edibility. This cell wall is composed primarily of cellulose, hemicellulose and pectin, and is broken down into simple sugars, or sugar alcohols, by a number of enzymes (Colinas-Leon and Young, 1981). The three heterogeneous cell wall compounds are hydrolysed by different cellulases, hemicellulases and pectinases (Awad and Young, 1979; Colinas-Leon and Young, 1981). These broad groups are then subdivided into specific function-based enzymes. For example, pectin methylesterase and polygalacturonase are two important pectinases in avocado metabolism. The ripening enzymes operate co-operatively to break down the cell wall and cause fruit ripening, and a deficiency in one of these enzymes will impair ripening.

A similar study was performed by Awad and Young in 1979 but only on a limited number of fresh fruit. In the last decade studies on ripening enzymes appear to have fallen out of favour, perhaps because of the laborious methods previously employed to monitor enzyme activity, but variable ripening or incomplete ripening which have been flagged as problematical, especially for ready ripe programmes due to the logistical problems caused, must be attributed to variation or low activity of one or more of these enzymes. While Blakey *et al.* (2009) were able to show that fruit water content at harvest is critical to the initiation of the ripening process, this constituted about 70% of the variation. Other factors relating to the actual ripening process, which implies enzyme action, may explain the other components of the variation. Thus, if better knowledge of the relative activities of the enzymes as affected by various conditions of environment and maturity are known, it may be possible to predict, manipulate or optimize enzyme expression/activity during and post storage to ensure better quality fruit for the consumer.

This study aims to monitor the change in these enzymes during ripening, such that a better understanding of the ripening process as affected by factors such as fruit origin and maturity is gained, and thereby help to create ways of changing or predicting fruit quality at final destination and thus enhance returns.

MATERIALS AND METHODS

Fruit were obtained approximately monthly from Tzaneen, Limpopo Province and Howick, KwaZulu-Natal from 2007-05-18 to 2008-08-13. Fruit were immediately sampled by removing a portion of mesocarp with a 15 mm cork borer, and plugging the hole with petroleum jelly to prevent desiccation and fungal infection. This was repeated five or six times until ripe. The samples were flash frozen in liquid nitrogen and freeze dried for storage until later analysis. Respiration and ethylene production of whole fruit were measured using an infrared gas analyser (CO_2) and gas chroma-

tograph (ethylene) equipped with a flame ionization detector and activated alumina column using the methods described in Blakey *et al.* (2009). Ripeness was measured using a densimeter. Sugars were assayed by the method of Liu *et al.* (2002). Total protein was assayed using the Bradford method (Bradford, 1976) after extraction using the method of Kanellis & Kalaitzis (1992) and enzyme activity was measured spectrophotometrically by measuring the optical density change in dinitrosalicylic acid (DNS) at 575 nm (Moodie, 2003; Al-Zuhair, 2008).

RESULTS AND DISCUSSION

Fruit from Tzaneen had a much larger ethylene climac-

teric than that from Howick, peaking between 400 and 700 µL/kg/h while fruit from Howick peaked at approximately 100 µL/kg/h (**Figure 1**). Fruit maturity as indicated by moisture content, did not appear to influence the result. No such difference was observed in the respiration rate (**Figure 2**), and no clear pattern relating to origin or maturity alone was discernable.

Fruit softening generally followed a linear pattern with an increase in the ripening rate as fruit mature (**Figure 3**). The softening rate of fruit from Howick increased from 1.7 to 2.0 to 3.1 densimeter units/d as fruit matured. Fruit from Tzaneen showed a similar trend. A rate of 2 units/d for non-stored fruit would appear to ensure softening within 14 days.

Protein synthesis showed a pattern similar to that of the ethylene climacteric (**Figure 4**). However, the protein contents of fruit which were highest at the time of harvest did not decline towards ripening. The pattern of changes measured for the major ripening enzymes
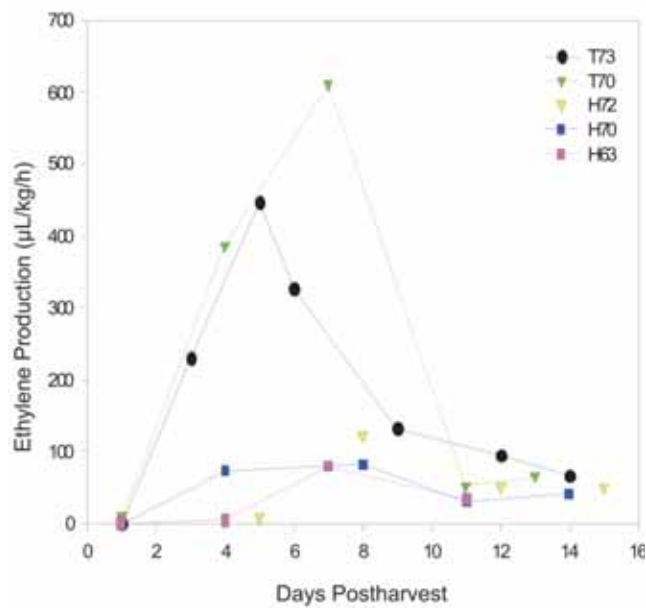

Figure 1. Ethylene production during ripening of fruit from Tzaneen (designated T) and Howick (designated H) for a range of maturities indicated as % moisture ranging from 63 to 73%

Figure 2. Respiration during ripening of fruit from Tzaneen (designated T) and Howick (designated H) for a range of maturities indicated as % moisture ranging from 63 to 73%
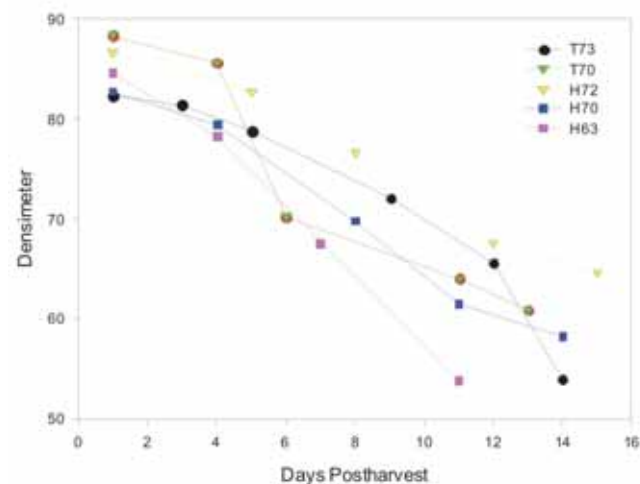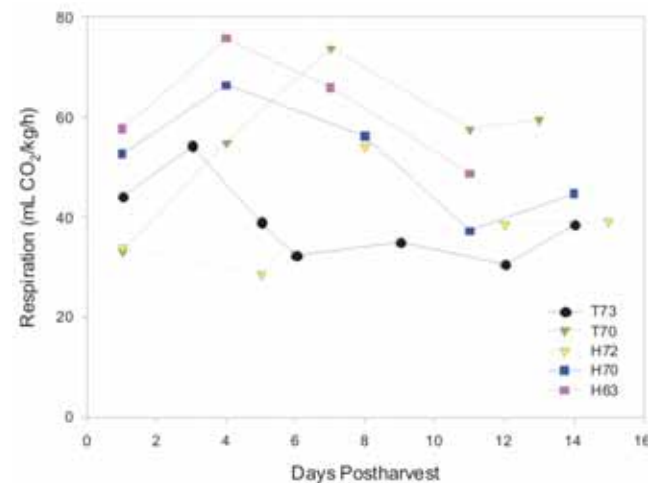

Figure 3. Densimeter readings during ripening of fruit from Tzaneen (designated T) and Howick (designated H) for a range of maturities indicated as % moisture ranging from 63 to 73%. A reading of less than 60 indicates "eating ripe"
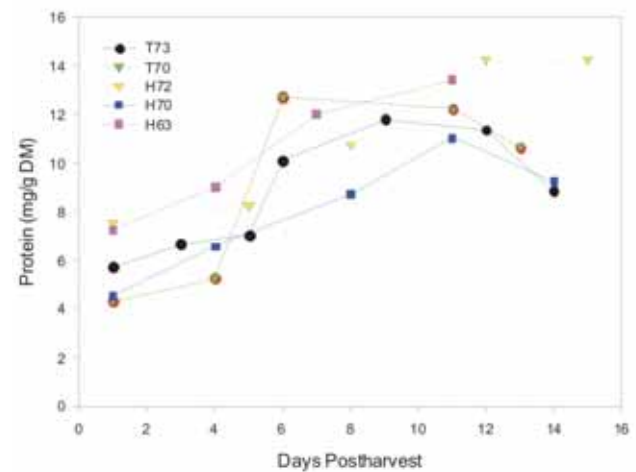

Figure 4. Protein concentration during ripening of fruit from Tzaneen (designated T) and Howick (designated H) for a range of maturities indicated as % moisture ranging from 63 to 73% for 5 harvests in 2007, from Tzaneen (T) and Howick (H) during ripening. Numbers in the caption refer to the moisture content

is shown in **Figure 5**, **6** and **7**. Pectin methylesterase (PME) prepares the pectin for degradation by de-esterifying pectin into pectic acid. As can be seen from **Figure 5** and **6**, the activity of polygalacturonase (PG) depends on the activity of the PME, the latter following the former, as PG can only degrade pectic acid, not pectin. PME has been shown to decline during ripening, and this is generally the case. It also appears that the higher the PME activity, the higher the PG activity. However, the late season fruit from Howick showed very low pectinase (PME + PG) activity but ripened the fastest. A possible reason for this is that the cellulase activity was higher throughout (**Figure 7**). The first harvest from Howick showed the greatest pectinase activity and high cellulase activity near ripeness. This implies that in determining the ripening of avocados, the complete suite of ripening enzymes, and not only certain

enzymes needs to be considered. A higher activity of one may compensate for lower activity of others, in terms of overall perception of ripeness as determined by softness.

It is suggested that avocado fruit require a certain amount of overall enzyme activity to ripen, and the energy for protein (enzyme) synthesis must be provided by stored sugars. The only sugars present in adequate amounts were mannoheptulose and its sugar-alcohol form perseitol. The other sugars (sucrose, glucose and fructose) were present in negligible amounts and showed no clear trend post-harvest. Mannoheptulose and perseitol showed a rapid decline in the first week post-harvest (**Figure 8** and **9**) as the protein concentration increased rapidly. It is suggested that those fruit with low initial protein content will need to use more energy to synthesise adequate amounts of the required ripening enzymes. If these energy re-
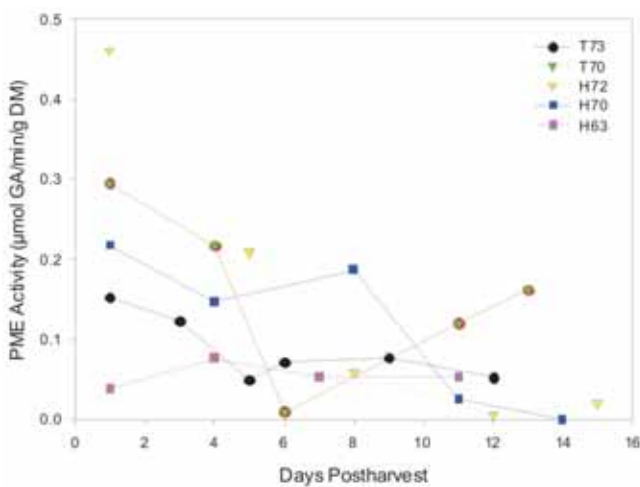

Figure 5. Pectin methyl esterase (PME) activity during ripening of fruit from Tzaneen (designated T) and Howick (designated H) for a range of maturities indicated as % moisture ranging from 63 to 73% for 5 harvests in 2007, from Tzaneen (T) and Howick (H) during ripening. Numbers in the caption refer to the moisture content
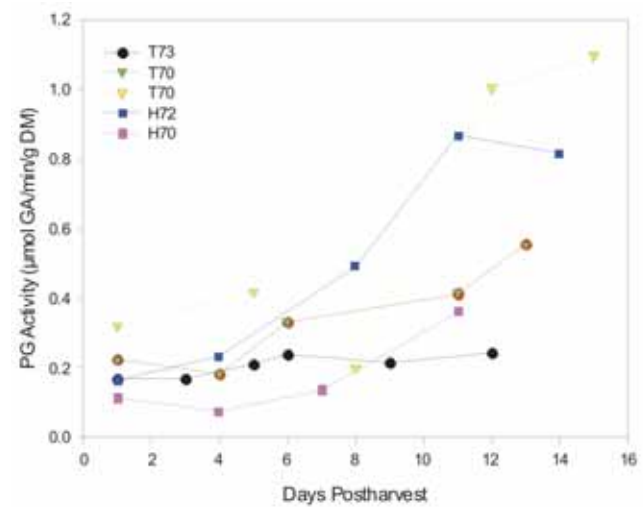

Figure 6. Polygalacturonase (PG) activity during ripening of fruit from Tzaneen (designated T) and Howick (designated H) for a range of maturities indicated as % moisture ranging from 63 to 73% for 5 harvests in 2007, from Tzaneen (T) and Howick (H) during ripening. Numbers in the caption refer to the moisture content
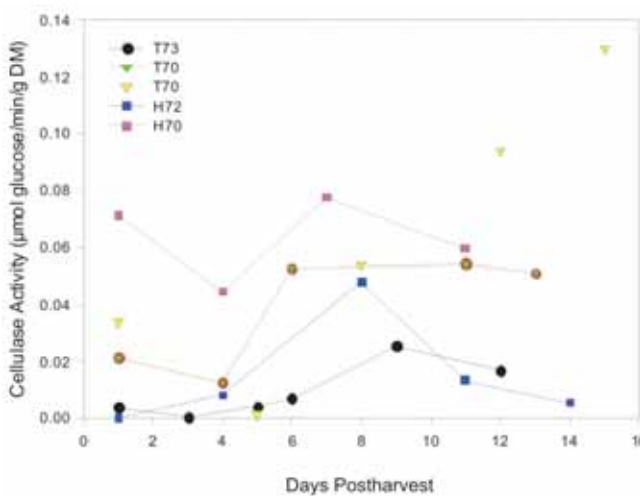

Figure 7. Cellulase activity during ripening of fruit from Tzaneen (designated T) and Howick (designated H) for a range of maturities indicated as % moisture ranging from 63 to 73% for 5 harvests in 2007, from Tzaneen (T) and Howick (H) during ripening. Numbers in the caption refer to the moisture content
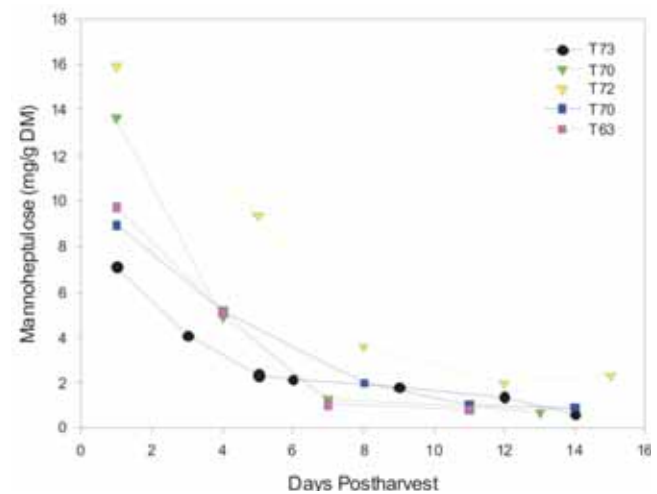

Figure 8. Mannoheptulose concentration during ripening of fruit from Tzaneen (designated T) and Howick (designated H) for a range of maturities indicated as % moisture ranging from 63 to 73% for 5 harvests in 2007, from Tzaneen (T) and Howick (H) during ripening. Numbers in the caption refer to the moisture content

serves are exhausted (as is evident in **Figure 8** and **9**) before the ripening process is completed, an alternative energy source would need to be used to sustain metabolism. This could come from protein breakdown and may be adverse for fruit quality. The inference is that initial total protein concentration together with sugar content, could be a useful maturity and/or quality marker. Protein concentration may be measurable using near-infrared spectroscopy because protein is present in appreciable amounts in avocado mesocarp. Mannoheptulose and perseitol can be measured in the same way. It is not yet known how storage temperatures or cold chain maintenance affects the production of the various enzymes, and the consequent effects on energy requirements or sugars, but indications from the research presently being conducted by the authors implies that sugar content, especially of the two mentioned sugars, is indeed important for the later fruit quality.

CONCLUSIONS

Avocado fruit show a general sequence of ripening with the respiratory climacteric a few days before or on the same day as the ethylene climacteric, followed by the protein climacteric approximately four days later. Peak PME activity occurred at harvest with cellulase activity peaking about a week later and PG activity a week after that. However, there were deviations from this pattern and it is suggested that the result may relate to the variable ripening found in consignments of avocados. The results clearly show variations in fruit from different production locations and across the season. Combining fruit from different orchards or farms is only going to exacerbate variable ripening in consignments of avocados, and helps to explain at least some of the variation in ripening and quality often found in the market.

As a baseline has been established for non-stored fruit, comparisons can be made with fruit that have been stored at 1ºC and 5.5ºC. Stored fruit typically ripen a week earlier than fresh fruit, so it is reasonable to expect that the ripening enzymes are synthesized during storage and very rapidly after removal from cold storage. The interaction with the storage environment is, however, not known. Nevertheless, the possibility of using the sugars and total protein content at harvest as markers for later ripening and quality prediction has been demonstrated.

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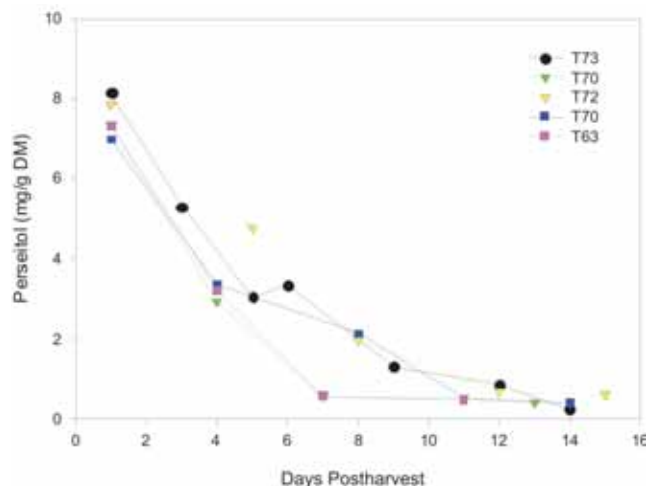
Figure 9. Perseitol concentration during ripening of fruit from Tzaneen (designated T) and Howick (designated H) for a range of maturities indicated as % moisture ranging from 63 to 73% for 5 harvests in 2007, from Tzaneen (T) and Howick (H) during ripening. Numbers in the caption refer to the moisture content